Putting together rather than taking apart

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meeting point

Putting together rather than taking apart

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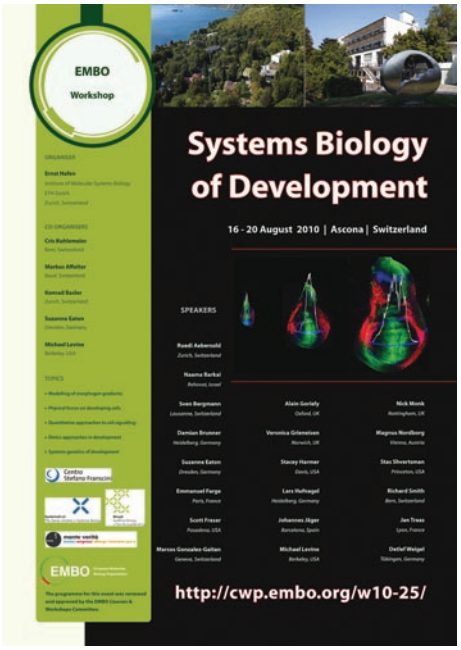
The town of Ascona in Switzerland, nestled on the northern shore of Lago Maggiore, hosted the 112 participants in the first systems biology meeting focused on developmental biology. The EMBO workshop was held between 16 and 20 August and brought together a multidisciplinary group of scientists who use systems approaches to understand how the size and shape of multicellular organisms and organs are determined.

As Denis Noble—one of the pioneers of systems biology—noted of this new research field, "it is about putting together rather than taking apart, integration rather than reduction." The relatively new approach of systems biology aims to integrate and analyse complex data from various experimental sources and to use interdisciplinary tools, with the long-term aim of developing predictive computer models of individual organisms. It was with these goals in mind that Ernst Hafen (ETH Zurich, Switzerland) and his co-organizers brought together scientists from different disciplines at the EMBO workshop on the 'Systems Biology of Development' to discuss and make progress on several topics, including technologies for systems biology, modelling morphogen gradients, quantitative approaches to cell signalling, transcriptional networks, physical forces on developing cells, cellular dynamics and the systems genetics of development.



The Babel-like multilingual communication at the meeting reminded me of a 1978 meeting at which *Drosophila* geneticists and molecular biologist joined together in Crete to combine techniques for a better understanding of gene function in development. Despite their different vocabularies, they soon realized the power of combining 'good old genetic' with molecular approaches. Within a few years they were able to speak the same language and harvest the fruits of their combined labour. The Ascona meeting was similar and we might not need to wait long for another rich harvest.

Technologies for systems biology

Although experimental and theoretical biologists have been navigating the human genome for almost ten years, a proteomic map is yet to be completed. Ruedi Aebersold's team (ETH Zurich) is working on this using sophisticated mass spectrometry (Picotti *et al,* 2010). His group presented fascinating technical advances and software that can generate and analyse reproducible and quantitatively accurate proteome databases. By using this targeted mass spectrometry, as few as four copies per yeast cell of some types of protein molecule can be detected. Erich Brunner (U. Zurich, Switzerland) and his collaborators reported a mass-spectrometry-based high-resolution imaging technique to identify and locate low-molecular-weight molecules *in situ* on the surface of tissues. These approaches make it possible to combine mass spectrometry and microscopy to provide five-dimensional resolution (time, space, and genome) with which to understand the developmental processes of multicellular organisms. The team has identified anterior–posterior compartment-specific components in *Drosophila* wing imaginal discs, with a focus on the boundary region—a signalling centre that organizes organ growth by positioning long-range signalling molecules.

Three other novel imaging approaches also received attention. Scott Fraser and his team (Caltech, CA, USA) introduced a new type of microscope that combines two-photon technology with light-sheet microscopy to provide higher resolution and less cellular damage—which are key to live imaging—than other microscopes. They hope to create labelling reagents—nanoparticles—with high photostability and brightness that will allow clear imaging at the single molecule level. One new development is the refinement of biosensors with sufficient sensitivity to monitor single-cell proteomic experiments.

Christof Aegerter (U. Zurich) reported a novel approach to fluorescence microscopy that uses scattered light for imaging with sub-wavelength resolution through optically thick turbid layers (Vellekoop & Aegerter, 2010).

Thomas Bürglin and colleagues (Karolinska Institutet, Sweden) have developed a multi-channel spatiotemporal microscopy framework to compose a numerical map describing the expression patterns and intensities of developmental control genes throughout *Caenorhabditis elegans* embryogenesis (Hench *et al*, 2009). Their approach seems likely to be applicable to other developing embryos and organs.

System gradients

The mechanisms by which the spatially graduated Decapentaplegic (Dpp) morphogen regulates both position-independent proliferation and patterning in the *Drosophila* wing imaginal discs were the focus of several presentations. Marcos Gonzalez-Gaitan's group (U. Geneva, Switzerland) quantified the Dpp concentration across the wing discs. Both Dpp concentration and signalling increase relative to wing-disc size, and mitosis commences when Dpp signalling has increased by 50% from the beginning of the cell cycle. The Dpp gradient also seems to flatten as the Dpp degradation rate decreases during development, contributing to the maintenance of increased signalling in cells. Proliferation control is mediated by temporal changes in the cellular levels of Dpp morphogen signalling. If the gradient is linked to tissue size during development, the proposed mechanism could generate position-independent growth rates. The group formulated a novel model for proliferation control during morphogenesis (Andrew *et al,* 2009).

The genes engaged in the interpretation of Dpp signalling have recently been identified. Konrad Basler and his collaborators (U. Zurich, Switzerland) and Petros Koumoutsakos (ETH Zurich) reported on the Fat pathway. Comparison between the cell proliferation rate under uniformly high Dpp or uniformly low Fat signalling activities revealed that Dpp and Fat regulate growth in the wing primordium in a complementary manner: in the periphery of the disc and in the centre, respectively. Basler and Koumoutsakos propose that Dpp and Fat signalling are regulated in parallel rather than hierarchically, and that a uniform proliferation rate along the anterior–posterior axis is achieved through their complementary effects on growth.

To understand the mechanisms of Dpp signalling, Markus Affolter and co-workers (U. Basel, Germany) and Sven Bergmann (U. Lausanne, Switzerland) used enhancers of Dpp target genes to investigate the scaling of the Dpp gradient. Giorgos Pyrowolakis and his collaborators (U. Freiburg, Germany and the Research Institute of Molecular Pathology, Vienna, Austria) conducted a whole genome *in silico* screen for evolutionarily conserved activating and silencing elements, by analysing the genomes of 12 species of *Drosophila*. They are now engaged in a large-scale transgenic analysis to find target genes involved in Dpp signalling and wing disc development.

Although the wing disc is probably the best understood developing organ and is ideal for studying gradient-controlled development, its analysis at molecular level is complicated. Progress will depend on the establishment of an *in vitro* culture system for imaginal discs. Researchers have worked towards this for decades, but progress has been slow and collaboration with engineers might be required to establish such a system.

The role of the auxin morphogen gradient in root and shoot vascular pattern development was discussed extensively. From his analysis of auxin and auxin transport proteins, Christian Mazza (U. Fribourg, Switzerland) elaborated a stochastic model to explain the formation of local auxin peaks and model plant growth (Fournier *et al*, 2009). Veronica Grieneisen (John Innes Centre, UK) presented experimental data and models to explain how the auxin morphogen gradient is established as a result of a 'reflux-loop' that precisely locates auxin transport proteins. She and her co-workers analysed the sub-cellular spatial dynamics of the RHO family of small G-polarity proteins and their relationship with the auxin morphogen gradient. They developed a model to explain how the auxin morphogen can trigger the spontaneous emergence of intracellular polarity and the resulting macroscopic tissue properties. They propose that cellular geometries, intercellular and intracellular gradients, as well as cell-polarity dynamics are interlinked and account for plant morphogenesis at the correct spatial and temporal timescales; a system befitting a systems biology approach.

Marta Ibanes and Ana Isabel Cano-Delgado (Centre for Research in Agricultural Genomics, Barcelona, Spain) discussed the role of auxin and brassinosteroids in shoot vascular patterning. From a quantitative analysis of shoot vascular patterning in a variety of *Arabidopsis* mutants, they developed a mathematical model to explain the mode of auxin action. This suggests that it is not the overall auxin level, but the periodic auxin maxima that position vascular bundles.

Chris Kuhlemeier (U. Bern, Germany) and co-workers reported that auxin accumulates in the shoot apical meristem at specific sites—by directional transport, mediated by auxin transport proteins—and initiates leaf formation. Localization of the auxin transport proteins to the plasma membrane is sensitive to osmotic changes. On the basis of elegant studies in which tension and pressure were modified by external force, they propose that membrane tension and turgor can regulate intracellular localization and function of the auxin transport proteins, including during organogenesis (Braybrook & Kuhlemeier 2010).

System signalling networks

Jose Ramon Dinneny (Temasek Lifesciences Laboratory, Singapore) reported that plants respond to salt stress with waves of transcriptional activity. These waves are associated with dynamic changes in growth rates and morphology. On the basis of these observations, his group has identified marker genes for use in high-throughput quantitative PCR experiments, to be followed by live-imaging analysis of roots. These methods can be use to follow changes in the transcriptional programme that can be correlated with the applied stress. They generated a spatiotemporal transcriptional map, following the salt-stress response in different tissue layers to understand the mechanisms that establish homeostasis after stress.

By combining mouse genetics with quantitative analysis and mathematical modelling, Javier Lopez-Rios (U. Basel, Germany) and colleagues uncovered a system of self-regulatory interlinked signalling feedback loops that control vertebrate limb development. They suggest the use of the mouse limb bud as a model in which to study organogenesis with a systems biology approach.

System transcriptional networks

In his inspiring talk, Michael Levine (U. California, Berkeley, CA, USA) reported work using elegant, whole-genome ChIP-chip, ChIP-seq and Gro-Seq assays to show that most crucial developmental-control genes contain paused RNA polymerase II before their activation during embryogenesis. By this mechanism, these genes are poised for rapid, synchronous activation, thus achieving transcriptional precision. High-resolution, quantitative *in situ* hybridization assays suggest that transcriptional repressors block the release of RNA polymerase II from the pause sites. Levine also presented evidence for the existence of 'shadow enhancers', which help to ensure robustness in gene expression in response to environmental and genetic fluctuations.

Bart Deplancke (Federal Polytechnic School of Lausanne, Switzerland) reported the

development of a gene-centred approach—the high-throughput gateway-compatible yeast one-hybrid system—to identify physical interactions between transcription factors and regulatory elements of interest. His group sequence-verified 630 (85%) of the predicted *Drosophila* transcription factors and generated a robotic platform that performs fully automated yeast one-hybrid screens.

Jay Parrish (U. Washington, WA, USA), in collaboration with the Lily and Yuh Nung Jan laboratory (U. California, San Francisco, CA, USA), described the molecular changes that accompany the developmental transition from rapid growth to scaling growth in the dendrites of the *Drosophila* peripheral nervous system. They identified developmentally regulated gene expression modules that correspond to the two growth phases of larval neurons. Future work will hopefully reveal how expression of the modules is coordinated and how motor neurons and their synaptic partners interact, as well as defining the link between transcriptional programmes and development.

Cellular dynamics

By combining real-time fluorescence imaging with automated, quantitative image analysis and computer simulations, Damian Brunner and colleagues (EMBL, Heidelberg, Germany) analysed the fascinating pulsing behaviour of amnioserosa cells during dorsal closure in *Drosophila* embryogenesis. The cell pulsing seems to be a force-generating mechanism (Solon *et al*, 2009). The group suggested that there are two forces in the process: (i) while changing shape, the amnioserosa cells that cover the dorsal opening exert a pulling force on the surrounding epidermal tissue; (ii) a supra-cellular band of contractile actin that surrounds the opening provides clutch-like activity that counteracts the epidermal relaxation, translating the amnioserosa-generated epidermal displacements into net dorsal-ward movement. Remarkably, their computer simulations suggest that an additional mechanism is needed to optimize and maintain the interplay of these forces to achieve dorsal closure. The prediction of unexpected factors is a pleasant feature of the recent models.

Emmanuel Farge (Institut Curie, Paris, France) described how the expression of the twist gene can be activated mechanically during *Drosophila* embryogenesis. His group uses magnetic tweezers *in vivo* to measure and apply forces that can modulate and mimic morphogenetic movements in living embryos (Fernandez-Sanchez *et al,* 2010). He also reported the mechanical activation of the expression of c-Myc, which initiates the programme of tumour progression in colon cancer in genetically predisposed mouse tissue. As pointed out by Stas Shvartsman (Princeton U., NJ, USA), the systems biology of development needs to re-invent classical experimental embryology and develop techniques that can quantitatively measure the responses of a large number of embryos or cells to precise manipulations.

The effect of mechanical forces on development is also a focus of Christof Aegerter's group's work. They have designed techniques to exert controlled forces on *Drosophila* wing discs and to measure their mechanical properties (Nienhaus *et al*, 2009), and have published an attractive model describing the relationship between mechanical stress and growth rates (Aegerter-Wilmsen *et al,* 2010).

Yanlan Mao (Cancer Research UK, London Research Institute) developed a computational model to describe epithelial tissue growth. She used Dachs, an atypical myosin that polarizes junctional tension and orients cell divisions by altering the cell geometry in the *Drosophila* wing imaginal discs. The polarized cell tension is sufficient to orient cell divisions and tissue growth. Planar polarization of Dachs is ultimately oriented by long-range morphogen gradients emanating from compartment boundaries, and is therefore a mechanism by which these gradients are linked to the control of tissue shape.

Systems genetics

Two excellent papers about systems genetics were presented at the workshop. Both seek to establish the relationship between phenotypic and genotypic variations, to model regulatory networks and to describe the link between phenotype, environment and evolution. Detlef Weigel (MPI for Developmental Biology, Tübingen, Germany) and his group use both forward genetic and genome-wide association approaches in *Arabidopsis* to rapidly map genetic variants and understand how they bring about phenotypic effects (Atwell *et al*, 2010). Their approach is particularly suited to analysis of the plant immune system, with its extreme allelic diversity. This approach might be used in future to better understand the nature of hybrid necrosis.

Magnus Nordborg (Gregor Mendel Institute, Vienna, Austria) and his group use inbred *Arabidopsis* lines and genome-wide association studies to describe many different traits in many different environments. Their aim is to identify genetic variants that have altered essential functions as environmental adaptations, and to then speculate about their evolutionary significance (Atwell *et al*, 2010; Todesco *et al,* 2010). Advanced DNA-sequencing technologies have enabled hundreds of *Arabidopsis* lines to be sequenced. This has provided an understanding of how genetic variation translates to phenotypic variation and how this depends on the environment. Such an approach might develop our understanding of evolution and has practical implications for agriculture and medicine. However, as Nordborg noted, "sequencing is cheap and thinking is expensive".

The meeting was an excellent demonstration of why taking a systems biology approach to multicellular systems is ideal for developmental biology. Bringing physicists, mathematicians, computer scientists and population geneticists to work together with developmental geneticists has already provided unexpected insights into biological systems, as highlighted by this meeting. Switzerland should be commended for its insightful systems biology initiative, SystemsX (http://www.systemsx.ch), which facilitates interdisciplinary projects and provided generous support for the meeting.

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